Annual Review of Genetics

Transgenerational Epigenetic Inheritance

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Annu. Rev. Genet. 2018. 52:21–41

First published as a Review in Advance on August 30, 2018

The *Annual Review of Genetics* is online at genet.annualreviews.org

https://doi.org/10.1146/annurev-genet-120417-031404

Keywords

epigenetics, transgenerational inheritance, germ cells, development

Abstract

Inheritance of genomic DNA underlies the vast majority of biological inheritance, yet it has been clear for decades that additional epigenetic information can be passed on to future generations. Here, we review major model systems for transgenerational epigenetic inheritance via the germline in multicellular organisms. In addition to surveying examples of epivariation that may arise stochastically or in response to unknown stimuli, we also discuss the induction of heritable epigenetic changes by genetic or environmental perturbations. Mechanistically, we discuss the increasingly well-understood molecular pathways responsible for epigenetic inheritance, with a focus on the unusual features of the germline epigenome.

INTRODUCTION

Transgenerational: transmitted from one generation of organisms to at least two generations of offspring

Faithful inheritance of phenotypes from one generation to the next is one of the linchpins of life and is mediated primarily by copying and transmission of an organism's genomic DNA. However, in addition to inheritance of the genome, maintenance of cell fate also depends on inheritance of epigenetic information, as John Gurdon's nuclear transfer experiments demonstrated conclusively that development does not proceed by progressive loss of genomic sequences (46). In addition to cell fate, many other traits are heritable during mitotic cell division in multicellular organisms: A classic example in mammals is X chromosome inactivation in females (85). Here, cells in the early embryo randomly choose one of two X chromosomes for silencing, a choice that is then remembered for many subsequent cell divisions—this somatic memory famously accounts for the large fur color patches in calico cats.

Mitotic inheritance requires that epigenetic information survives the twofold dilution of cellular contents that occurs every cell division. Implicit in the fact that all the distinct cell types in the body are reestablished in each organismal generation, the vast majority of cell state information is erased, or reprogrammed, in the germline of multicellular organisms. Yet, decades of study have revealed a wide range of special cases of so-called transgenerational epigenetic inheritance from one organismal generation to the next. Here, we review a variety of paradigms for germline transmission of epigenetic information, survey unusual features of the germline epigenome, and finally discuss the potential for epigenetic marks to transmit environmental information from ancestors to future generations.

MAJOR EPIGENETIC INFORMATION CARRIERS AND EPIGENETIC CROSSTALK

Genetic and molecular studies of both mitotic and transgenerational epigenetic inheritance paradigms have identified a set of key pathways involved in epigenetic gene regulation: (*a*) transcription factors (107), (*b*) chromatin architecture (1), (*c*) covalent DNA modifications (35), (*d*) small RNAs (19, 39), and (*e*) prions (53). Sequence-specific DNA-binding proteins are the primary determinants of cell fate in multicellular organisms (141), and prion-mediated inheritance is well established as an epigenetic inheritance paradigm in fungi (53, 128). However, there is at present little evidence for germline transmission of TF levels or prion states in multicellular organisms; therefore, we focus on chromatin, DNA modification, and small RNAs. We start by briefly reviewing the current understanding of the copying mechanisms for each of these pathways, then turn to the unusual features of the germline epigenome. Importantly, the vast majority of epigenetic marks—cytosine methylation, histone modifications, etc.—across the genome are not efficiently inherited on their own. Instead, maintenance of epigenetic information is often reliant on interactions between at least two distinct information carriers, as discussed below.

Chromatin architecture comprises the nucleoprotein packaging state of eukaryotic genomes, composed of repeating nucleosomes. It has been implicated in a wide range of epigenetic phenomena, including cell fate maintenance. Genetic screens in *Drosophila* for mutations that affect cell fate memory uncovered the Polycomb and Trithorax factors, which encode enzymes involved in remodeling chromatin structure (124). The mechanism by which chromatin states are replicated is incompletely understood and remains the subject of a great deal of ongoing research (1, 71). In broad outline, parental histones are distributed to both daughter chromosomes during replication and are retained close ($\pm$400 bp) to the locus from which they were evicted. At a small number of genomic loci, the newly synthesized histones that fill in the gaps between parental nucleosomes are then decorated to match the covalent modification states of the "old" nucleosomes. Key to this copying mechanism is the fact that many modifying enzymes bind to the very modification that

they catalyze (e.g., H3K9 methyltransferases are activated by nucleosomes with K9-methylated H3 tails) (25). However, such feedback cannot on its own account for copying of histone modification patterns, as the vast majority of histone modifications are rapidly erased/diluted following removal of an inciting stimulus, whether an environmental signal for gene activation or a *cis*-acting feature such as a silencer element (30, 150). An emerging theme is that copying chromatin states often requires the juxtaposition of weak feedback that maintains histone marks for only a few cell divisions on its own, with the continued presence of an instructive input such as a silencing element (150) or local production of RNAs that recruit/activate chromatin regulators (59).

Methylation of cytosine at the 5 position is a widespread (but not universal) mechanism central to many epigenetic inheritance paradigms. Among the major systems for epigenetic inheritance, the copying mechanism for cytosine methylation patterns is conceptually the simplest. Heritable cytosine methylation primarily occurs in the context of the symmetric CpG dinucleotide, where replication results in two daughter genomes each carrying a hemimethylated CpG that provides a substrate for the maintenance methyltransferase (Dnmt1 in mammals, Met1 in *Arabidopsis*). Plants also methylate cytosines in other sequence contexts (CHH and CHG), but this generally requires ongoing reestablishment by small RNA or heterochromatin-directed methylation pathways (35, 138) and so is not heritable on its own. CpG methylation patterns can be stably inherited in dividing mammalian cells, but they are largely erased from one organismal generation to the next. By contrast, methylation epialleles in plants can be inherited for hundreds of organismal generations (104, 108). In addition to cytosine methylation, recent studies have implicated another DNA modification—adenine 6-methylation—as a potential carrier of epigenetic memory (84). In this case, there is no clear mechanism for copying m6A patterns; we anticipate rapid advances in understanding the function and targeting of this modification in the coming years.

The regulatory molecules broadly lumped together under the term small RNAs encompass a wide variety of distinct entities that differ both in their biogenesis and in their mechanism of action, and include well-studied classes such as microRNAs as well as more recently characterized and poorly understood entities such as transfer RNA (tRNA) fragments (39, 54, 57). Small RNAs—particularly small interfering RNAs (siRNAs) and Piwi-interacting RNAs (piRNAs)—are central to many of the best-established transgenerational epigenetic inheritance paradigms such as paramutation and RNA interference (RNAi). In these systems, small RNA levels are typically maintained by a mechanism involving RNA-dependent RNA polymerase (RdRP): Small RNAs prime RdRP-dependent copying of longer host transcripts, then these transcripts are processed to produce various secondary RNA species. Mutation of RdRP-encoding genes prevents stable epigenetic silencing in many systems, and stable transgenerational inheritance systems are seldom found in species that do not encode an RdRP (including flies and mammals).

Although we focus on copying mechanisms for each epigenome in isolation, epigenetic inheritance paradigms generally rely on interplay between two or more of the various epigenetic pathways described above. For example, in both plants and mammals, small RNAs can direct de novo cytosine methylation at homologous genomic loci (7, 158). Similarly, small RNAs can direct formation of H3K9-based heterochromatin (148), whereas long noncoding RNAs play complex roles in recruitment and modulation of both H3K27/Polycomb and H3K4/Trithorax chromatin pathways (116). In turn, heterochromatin and DNA modification can affect expression of small RNA–generating loci. As one prominent example, heterochromatin plays a key role in directing proper expression and processing of piRNA precursor transcripts in flies (76).

Epialleles: different states of the same genomic locus that differ in their associated epigenetic marks

Piwi-interacting RNAs (piRNAs): a class of small RNAs expressed during germline development in mammals, defined by their association with a Piwi-clade Argonaute protein

Paramutation: a special case of non-Mendelian inheritance in which a recessive paramutable allele is converted, in the presence of a dominant paramutant allele, to the dominant form

THE GERMLINE EPIGENOME

Central to understanding the mechanistic basis for transgenerational inheritance is a detailed accounting of the unusual features of epigenetic marks in germ cells. Although a common feature

of the germline epigenome is the widespread erasure/reprogramming of epigenetic marks, long-term inheritance of epialleles depends on at least some loci escaping from this erasure process. By contrast, signal-mediated editing of the epigenome could potentially program intergenerational effects of environmental conditions. Here, we briefly review germline-specific features of chromatin structure, cytosine methylation, and small RNAs.

Intergenerational: affecting the next generation; often used in opposition to the term transgenerational, which implies multiple generations of inheritance

Zygotic genome activation (ZGA): the process by which embryos begin transcribing their genome and thereby transition from reliance on maternally supplied transcripts

Chromatin

Nowhere is the contrast between somatic cells and gametes more pronounced than in chromatin packaging, particularly for sperm. In many organisms, the majority of histones are removed during spermatogenesis, and the genome is instead packaged with highly basic proteins such as protamines, allowing for supercompaction of the genetic material in the sperm head. Even in species that maintain nucleosomal packaging of the sperm genome (such as zebrafish or *Arabidopsis*), these nucleosomes are primarily comprised of germ cell–specific histone variants rather than the canonical histone proteins (61, 100). Not only is germline chromatin broadly distinctive in terms of nucleoprotein composition, but this packaging is quite heterogeneous across the genome. For example, in *Caenorhabditis elegans*, histone H3.3 is excluded from the X chromosome of both sperm and eggs (102), and in mammals, H3.3-containing nucleosomes are enriched at the promoters of early developmental regulators (34). Histone modifications also exhibit germline-specific patterns, with chromosome-wide H3K9 methylation accompanying sex chromosome inactivation in mammals and worms (74).

Chromatin architecture is less distinctive in oocytes than in sperm—the oocyte genome remains associated with nucleosomes—yet chromatin packaging in oocytes also differs substantially from that of somatic cells. As with sperm, oocyte chromatin also undergoes widespread histone replacement, and the resulting chromatin is characterized by germline-specific histone variants, such as H1oo in mammals (101). Histone modification patterns also differ markedly between oocytes and somatic cells. For example, the activation-related H3K4me3 modification, which is typically narrowly confined to the 5′ ends of transcribed genes, spreads across entire coding regions in mammalian oocytes (51).

Upon fertilization, these unusual chromatin architectures are extensively remodeled, and maternal and paternal genomes remain distinctively packaged for several cell divisions (8). The sperm genome is rapidly stripped of its packaging proteins, which are replaced with nucleosomes carrying the replication-independent histone variant H3.3. This replacement process is essential: In *Drosophila*, H3.3 is required at fertilization for decompaction and subsequent replication of the paternal genome (18); in *Arabidopsis*, mutants in the replication-independent H3.3 variants *HTR4* and *HTR5* exhibit delayed activation of the paternal genome (11); and in mammals, H3.3 is required for pericentric heterochromatin formation on the paternal pronucleus (119) and, intriguingly, for nuclear pore formation (63, 157). Early embryonic chromatin dynamics are also central to the process of zygotic genome activation (ZGA), which occurs as the embryo transitions from reliance on maternally supplied transcripts to transcription of its own genome. This process occurs at very different stages of development in various organisms. The major wave of ZGA occurs during the two-cell stage in mouse, but only after 12 cleavages, at the mid-blastula transition, in *Drosophila*. Evidence from multiple systems identifies histone provisioning by the oocyte as the global repressor of ZGA. In organisms with relatively late ZGA, a large supply of excess histones in the oocyte is successively diluted with every cell cycle until insufficient histones remain to fully package the genome, resulting in relief of global genome repression (3).

Although sperm chromatin is almost completely remodeled after fertilization, a subset of genomic loci may escape complete removal of their packaging proteins. Sperm centromeres often

retain their CenH3-containing nucleosomes even when the rest of the genome gets repackaged into protamines, and in some cases, these may be responsible for establishing centromeric identity on paternal chromosomes in the embryo (61, 115). Not only do some aspects of germline chromatin potentially escape erasure/remodeling, but germline chromatin can also direct persistent molecular changes in the embryo via crosstalk with other epigenetic marks, or via effects on early embryonic gene activation. As a key example of such crosstalk in mammals, Tet-mediated erasure of cytosine methylation is blocked at loci packaged into H3K9-methylated nucleosomes via their recruitment of the Tet inhibitor Stella (97). Embryonic gene control is also under the control of germline epigenetic information, as H3K27 methylation on the oocyte genome represses early gene expression in a wide range of organisms. Given the vast array of covalent histone modifications and histone isoforms as well as the challenges associated with chromatin mapping studies in low cell numbers, much remains to be learned about how germ cells are packaged and about chromatin dynamics during early embryogenesis.

Cytosine Methylation

When present in an organism, cytosine methylation patterns differ greatly between male and female gametes (35, 73). In mammals, the sperm genome is heavily methylated, and focal hypomethylation at CpG islands breaks up the otherwise nearly uniform landscape of methylation. Methylation in the oocyte exhibits far greater variance across the genome, as relatively long (approximately 100 kb) blocks of hypomethylated DNA are found over highly transcribed genes (78, 135). In contrast to animals, where the germline is set aside early in development and methylation landscapes are extensively reprogrammed (48), somatic patterns of cytosine methylation largely (but not completely) persist in plant germ cells. For example, in *Arabidopsis*, somatic CG and CHG methylation patterns are maintained in the sperm nuclei in pollen, whereas only CHH-context RNA-dependent DNA methylation at retrotransposons is erased (24). Indeed, the lack of substantial cytosine methylation reprogramming during gametogenesis is at least partly responsible for the unusual stability of epialleles in various plant species. Interestingly, in contrast to the relatively subtle cytosine methylation dynamics in plant germ cells, companion cells to the gametes exhibit distinctive methylation behaviors. For example, global demethylation in the central cell of ovules, which gives rise to the placenta-like endosperm, is responsible for revealing imprinted gene expression specifically in this tissue (see below).

Upon fertilization, germline methylation patterns are rapidly remodeled in most organisms. In mammals, this manifests as rapid and near-global active demethylation of the paternal genome, alongside slower demethylation of the maternal genome. By the blastocyst stage, the genome is globally hypomethylated, in both the inner cell mass and trophectoderm lineages. During implantation, remethylation proceeds distinctly in the epiblast—where methylation patterns typical of somatic tissues are reestablished—and in the extraembryonic ectoderm, characterized by widespread partially methylated regions (136). Curiously, reprogramming the germline methylome following fertilization in zebrafish is to some extent the inverse of that in mammals, as neither genome undergoes global demethylation. In fact, the maternal genome becomes hypermethylated during early cleavage divisions to match the sperm profile (68, 106). In seed-bearing plants, cytosine methylation changes are modest in the embryo. CHH methylation, absent in pollen, is restored to the paternal genome (24), and any loss of methylation in the egg cell (for which whole-genome methylation maps do not yet exist) is rapidly reversed. Interestingly, analogous to the hypomethylation observed in mammalian placenta relative to the epiblast, the endosperm exhibits widespread hypomethylation resulting from active Demeter-driven demethylation in the central cell that accompanies the egg cell (38, 58, 73).

Argonautes: proteins that have key roles as effectors in a variety of small RNA pathways; they bind to small RNAs including microRNAs, siRNAs, and piRNAs and are guided to targets via complementary base pairing

In animals, the global de/remethylation cycles that occur in primordial germ cells and in the early embryo serve to almost completely erase ancestral methylation patterns (47), presenting a significant obstacle to purely cytosine methylation-based epigenetic inheritance. That said, a small subset of loci somehow resist the global erasure of cytosine methylation that occurs upon fertilization, presumably thanks to H3K9me2-mediated recruitment of the Tet inhibitor Stella (97). These escapers tend to be located in or near relatively young transposons that retain the potential for continuing transposition, such as intracisternal A particle (IAP) endogenous retroviruses in mouse (135) and L1PA elements in humans (45). These escaping loci have drawn considerable attention, as they represent good candidates for potential intergenerational transmission of cytosine methylation patterns. Beyond such escapers, methylation epialleles could also in principle be maintained via other epigenetic placeholders (e.g., chromatin state or small RNAs) that could reestablish ancestral methylation states.

Small RNAs

In addition to somatic small RNA pathways (e.g., microRNAs), sexually reproducing organisms also carry a germline-specific RNA-based system for self/nonself discrimination. In animals, small RNAs in this pathway are known as piRNAs on the basis of their association with Piwi-clade Argonaute proteins (87). Plants lack a Piwi-clade Argonaute, but related genome defense functions are served by antitransposon siRNAs. The function served by the piRNA system is clearest in fly oocytes, where piRNAs are derived from various repeat elements via a complex biogenesis pathway initiated by nuclease digestion of long transposon transcripts (6, 20, 144). Mutations that affect the piRNA machinery release transposons from transcriptional repression, and resulting transposon mobilization causes extensive genomic damage and consequent sterility in most animals. However, piRNA systems differ dramatically in other organisms. For example, in *C. elegans*, the equivalent germline RNAs—the 21U-RNAs bound by Piwi protein PRG1—are unique rather than being derived from repeat elements and are transcribed individually as short (26 nt) precursors rather than being processed from a longer host transcript (12, 16, 43). PRG1 and its cargo play only minor roles in endogenous transposon control—they are responsible for repression of the DNA transposon Tc3—but, nonetheless, are involved in self/nonself recognition, as they are required for heritable silencing of transgenes (10, 23, 81, 130). The diversity of sequence space that can be surveilled by the vast repertoire of 21U-RNAs allows these species to recognize an arbitrarily complex transcriptome—21U-RNAs (and secondary 22G-RNAs) provide a protective memory of so-called healthy germline gene expression and subsequently recognize and silence novel sequences that are not protected by this memory system. Other species exhibit hybrid piRNA systems. For example, both transposon-derived and unique piRNAs are produced in two major waves during mammalian spermatogenesis (82, 151).

Although piRNAs are the most characteristic RNA pathway in germline development, mature germ cells often carry additional, or entirely distinct, RNA cargo. In mammals, for example, piRNAs are almost completely absent from ejaculated sperm, which instead carry a cargo comprised primarily of tRNA fragments, along with a smaller population (approximately 10–20% of small RNAs) of microRNAs (105). Although not completely understood, this dramatic epigenetic reprogramming event occurs during post-testicular maturation in the epididymis, where small RNAs produced in the epithelium appear to be trafficked to maturing sperm in exosomes (129). The cargo of sperm and oocytes is quite distinct, as the predominant small RNAs in oocytes are microRNAs as well as siRNAs derived from convergently transcribed genes (140).

Functionally, small RNAs in the germline can affect subsequent generations either directly via delivery to the zygote, or indirectly by directing chromatin or DNA modifications during

gametogenesis. For example, given that piRNAs can target cytosine methylases to the genome (7), environmental or genetic perturbations to the piRNA repertoire could affect offspring phenotypes via altered methylation landscapes. However, functions served by gametic small RNAs upon delivery to the zygote are not as well studied as their functions during gametogenesis, in part owing to the challenges inherent in obtaining large numbers of synchronized early embryos in most species. Naturally, small RNAs in the egg cell, versus those in the far smaller sperm cell, are more likely to function in the zygote. For example, in *Arabidopsis*, egg cells carry 24-nt-long RNAs that direct H3K9 methylation and nonCG cytosine methylation over targeted coding regions, thereby delaying activation of genes on the paternal genome (11). In flies, piRNAs in oocytes are required to prevent hybrid dysgenesis, in which offspring of crosses between two strains—one carrying a specific transposon, the other naive to this transposon—are sterile if the transposons are transmitted paternally, but protected from transposon mobilization if the transposon DNA is transmitted along with a protective complement of piRNAs in the oocyte (21). In contrast, the male gamete in many organisms is typically believed to carry little or no functional RNA to the zygote, as illustrated by hybrid dysgenesis. That said, there are several clear examples in which sperm RNAs appear capable of influencing early development, in some cases dramatically. For example, mouse embryos generated using *dicer*$^{-/-}$ conditional knockout sperm exhibit defects in preimplantation development that can be rescued by microinjection of sperm RNAs into the zygote (155). In *C. elegans*, transgenerational RNAi is efficiently transmitted through the male germline (2), although the relevant epigenetic marks transmitted in sperm could plausibly be histone modifications rather than small RNAs. In summary, the oocyte carries the majority of relevant regulatory RNAs that function in the early embryo of most species. However, there is substantial and increasing evidence that sperm also carry a functional RNA payload. Much remains to be learned about the complete RNA cargo of both gametes and about the functions of small RNAs in the early embryo.

Common Features of the Germline Epigenome

Despite the dramatic differences among germline epigenetic inheritance systems in various organisms, several features are common, if not universal, to these systems. Most generally, epigenetic inheritance pathways typically play central roles in self/nonself discrimination, and loss of the various epigenetic repression systems commonly results in transposon derepression and mobilization. Epigenetic marking systems presumably evolved primarily to serve this function, but these molecular pathways have also been co-opted for other functions. For example, genomic imprinting in mammals (below) utilizes cytosine methylation and chromatin-based repression to control expression of growth factors and other unique genes.

Another characteristic feature of the germline epigenome is the dynamic erasure/reestablishment of the epigenome every generation. This is particularly clear in the male germline: In mammals, cytosine methylation is erased twice every generation (in primordial germ cells and in the early embryo), histones are nearly completely eliminated during spermatogenesis, and small RNAs including piRNAs are eliminated during post-testicular maturation of sperm. By contrast, in plants, cytosine methylation patterns are largely maintained in the egg and sperm nuclei, which presumably accounts for the stability of their cytosine methylation epialleles. Interestingly, more dramatic epigenetic remodeling occurs in germline companion cells in plants: Loss of H3K27 methylation in the vegetative nucleus of pollen results in derepression of repeat elements and subsequent production of antitransposon 21-nt siRNAs (122, 134), whereas cytosine demethylation in the central cell of female gametes results in a globally undermethylated genome in the endosperm, which contributes to imprinted gene expression in this tissue (73).

INTER- AND TRANSGENERATIONAL INHERITANCE SYSTEMS

Epivariation: phenotypic or molecular variability between genetically identical organisms that is distinguished from molecular noise or phenotypic plasticity in that it is potentially heritable

Differentially methylated region (DMR): a genomic locus that can exhibit distinct cytosine methylation levels under different states or conditions

How does germline transmission of epigenetic information affect future generations, and how persistent are epigenetic states over time? A distinction is often made between intergenerational and transgenerational epigenetic inheritance (44, 95). In the former, epigenetic marks in the germline affect the following generation but are not copied/maintained in the subsequent germline. For example, in imprinted gene regulation, sex-specific programming of germline epigenetic marks results in parent-of-origin-dependent gene expression that is consistent across individuals in a population but is reset every generation. In contrast, more stable cases of epivariation such as paramutation, where two distinct phenotypes—green versus purple pigmentation in maize, most famously—can be stable for hundreds of generations or more. Here, we discuss representative examples of programmed epigenetic inheritance and long-term epivariation. We then turn to inter- and transgenerational effects of genetic or environmental perturbations on future generations.

Programmed Germline Epigenetics: Imprinting

Imprinting—parent-of-origin-specific expression of one of two alleles in a diploid organism, largely confined to species where the developing embryo enjoys ongoing maternal provisioning after fertilization—is a classic example of epigenetic inheritance (14, 93, 125). In the most famous example of imprinting genetics in humans, individuals carrying a heterozygous deletion for chromosome 15q11–13 can exhibit two dramatically distinct phenotypes: Individuals affected with Prader-Willi syndrome are obese and hypogonadal, whereas Angelman syndrome patients are characterized by ataxia and inappropriate smiling and tongue-thrusting. Although caused by the same genetic lesion, these patients differ in whether the deletion was inherited from the father's or mother's side—in other words, each copy of this locus remembers from which parent it came. In mammals, many imprinted genes are involved in placental development or more broadly in maternal provisioning. This observation motivates the compelling hypothesis that imprinted gene control often results from conflicts between mothers and fathers regarding resource allocation to offspring: Paternally expressed genes tend to drive increased provisioning to offspring, whereas maternally expressed genes prevent excessive investment in any one child (49, 50).

In mammals, imprinted genes occur in clusters surrounding a differentially methylated region (DMR), whose methylation status controls local gene expression. Although the majority of such imprinting control regions are methylated on the maternal allele and unmethylated on the paternal allele—thanks to the near-complete demethylation of the paternal genome following fertilization—several loci such as the *Dlk-Dio* locus escape this erasure process and exhibit the converse behavior. Other *cis*-acting epigenetic marks besides cytosine methylation can preserve parent-of-origin memory. In plants, most cases of imprinted gene expression rely on allele-specific differences in the repressive H3K27 methylation mark (73, 110), and oocyte-derived H3K27 methylation has also been implicated in certain cases of imprinting in mammals (62).

Much of our understanding of germline epigenetic dynamics comes from the study of imprinting control regions (142), which exhibit clearly distinct epigenetic states in male and female gametes and are epigenetically reset each generation during germline development. Although imprinting has been a central model system for understanding the dynamics of the germline epigenome, obligatory erasure and reestablishment of imprinted epigenetic marks every generation make imprinted genes poor candidates for transgenerational epigenetic information transfer. That said, environmental or genetic perturbations that disrupt epigenetic dynamics at imprinted loci could potentially affect offspring phenotypes.

Transgenerational Epivariation

Genetically identical organisms can exhibit a great deal of phenotypic variability. The majority of this variation, whether due to molecular noise or phenotypic responses to the environment, is not transmitted to the next generation. Yet a small subset of phenotypic differences can be inherited, with both Mendelian and non-Mendelian patterns of inheritance having been observed.

Paramutation. The earliest recognizable descriptions of transgenerational epigenetic inheritance come from non-Mendelian inheritance patterns in plants. Perhaps the first such report was made by Bateson & Pellew in their study of rogues in the garden pea (15). This phenomenon was later termed paramutation in Brink's studies in maize (22). Paramutation describes a behavior in which a trait appears dominant in an initial cross, but where resulting offspring that would be expected to be genetically heterozygous (Aa) instead behave as AA in subsequent crosses. In other words, the dominant allele appears to convert the recessive allele into a dominant one in *trans* (9). The best-studied case of paramutation comes from the study of maize pigmentation where a dominant paramutant *B′* allele (exhibiting transcriptional repression of the *B* transcription factor) converts its paramutable *B-I* homolog in *trans* to form a new paramutant *B′* allele (designated *B′**) (9). Similar phenomena are widespread in plants (132). Although far less common in animals, weak paramutation-like behavior has been described for a handful of loci in flies (55) and mammals (113).

Mechanistically, repression of the paramutant *B′* allele in maize requires all three major epigenetic information carriers, as mutants in various RNAi, cytosine methylation, and chromatin repression pathways all exhibit *b1* derepression. A key *cis*-acting feature of paramutable *b1* alleles is the presence of seven tandem 853-bp repeats upstream of the *b1* coding region. *b1* alleles lacking these repeats cannot be converted to the repressed state even in the presence of a repressed paramutant *B′* allele (137). Following confrontation of an active paramutable allele with a repressed paramutant allele, repeat-derived small RNAs (rather than, say, direct contact during homolog pairing) appear to mediate communication in *trans* between copies of the *b1* locus.

Mendelian inheritance of epialleles. Paramutation was discovered from investigation of a non-Mendelian inheritance pattern. However, this is relatively unusual, and many more cases of heritable epivariation have been described in plants in which silent and active alleles of a given gene coexist and segregate in a Mendelian manner during meiosis. Famous plant epialleles include the peloric variant of Toadflax, which is associated with cytosine methylation of the *Lcyc* gene, and genetically recessive *Cnr* (colorless, nonripening) mutants in tomato resulting from methylation of the *LeSPL-CNR* locus (108). Here, as with many other epialleles, the affected locus is located adjacent to a transposable element from which cytosine methylation spreads in *cis*.

A particularly well-studied example of epivariation is the *clark kent* alleles of the *SUPERMAN* locus in *Arabidopsis*. *SUPERMAN* encodes a transcription factor involved in flower development, and *sup* mutants exhibit overproduction of stamens and carpels. Jacobsen & Meyerowitz obtained multiple alleles exhibiting phenotypes similar to the *sup* mutant, which they dubbed *clark kent* (*clk*) mutants (67). *clk* alleles result from cytosine methylation and repression of the *SUP* gene. These alleles mapped to the *SUP* locus, did not result from sequence changes to *SUP* or its regulatory regions, and could be mimicked by manipulating DNA methylation. In contrast to paramutation, the hypermethylated allele segregates in a Mendelian manner and is fairly stable, reverting with approximately 1–3% frequency. Many similar examples have been described in *Arabidopsis*, and recent genome-wide surveys have identified thousands of DMRs across wild isolates (72) or across (epi)mutation accumulation lines propagated by single-seed descent for 30 generations (121). In

the latter study, Schmitz et al. (121) revealed overall stability of methylation patterns—methylation status of DMRs in descendant lines was generally correlated with the methylation observed in the relevant ancestral line. Along with this relatively stable propagation of methylation patterns, the authors identified widespread epimutations, defined as spontaneously occurring DMRs that could not be explained by genomic changes (single-nucleotide polymorphisms, transposon insertions, etc.). Heritable epialleles are thus common in plants, and the contribution of such epialleles to natural phenotypic variation, and to evolution, is an active area of interest (56, 153).

Long terminal repeat (LTR): a sequence repeat that is found at both ends of a wide variety of retroviruses (such as HIV) or endogenous retroviruses

Epivariation in mammals: the A^{vy} model. The majority of clear-cut examples of stable epialleles have been described in plants. This fact is variously rationalized as being related to either the lack of early segregation of dedicated germ cells or the sessile nature of plants that must therefore adapt to local conditions without the ability to change environments. Although stable transgenerational epialleles seem to be rare in mammals, researchers have described a few epialleles that exhibit partially penetrant inheritance over one or sometimes two generations.

The best-studied epivariable locus in mammals is the agouti viable yellow (A^{vy}) allele in mouse, which has been used extensively as a sensitized reporter for intergenerational effects of various environmental and genetic perturbations (see below). A^{vy} results from the insertion of an IAP retrotransposon upstream of the agouti (*A*) gene. The long terminal repeat (LTR) of the IAP acts as an alternative promoter and, when active, drives ectopic and continuous expression of the agouti gene, resulting in yellow coat coloration along with a variety of metabolic phenotypes. Inbred colonies of A^{vy} mice display a range of phenotypes from fully yellow and obese to pseudoagouti and lean, and these phenotypes are correlated with the extent of cytosine methylation spreading around the IAP element. Even though these animals are isogenic, or nearly so, the differences in their phenotypes are partially heritable. Agouti mothers bear litters that are disproportionately agouti, whereas brown mothers bear litters that skew brown (96). Coat color is transmitted only maternally, indicating that paternal reprogramming at this locus is complete, and embryo transfer experiments show that coat color is transmitted via oocytes (as opposed to resulting from intrauterine environmental effects). The mechanism(s) protecting the maternal, but not paternal, A^{vy} locus from methylation reprogramming in the germline remain unclear, although it is striking that IAP elements are among the rare loci that escape complete methylation erasure in primordial germ cell development and in early embryos.

Metastable epialleles in other model systems. Beyond the examples described in plants and mammals, a mechanistically diverse array of metastable epialleles has been identified throughout the tree of life. The pathways responsible for epigenetic inheritance differ between systems. For example, the [psi-] and [PSI+] phenotypes in budding yeast represent different protein-folding states of the Sup35 protein and do not rely on chromatin, DNA modification, or small RNA pathways for transmission. In contrast, prion-based inheritance seems to be rare in germline inheritance systems in multicellular organisms. Various evolutionary pressures—co-option of certain epigenetic pathways for essential gene regulatory functions, the spectrum of selfish genetic elements confronting the organism—presumably shape the repertoire of defense mechanisms that predominate in a given species.

In addition to many of the classic epialleles that drive gross morphological/coloration differences among organisms, it is certain that many more epivariable loci that have more subtle phenotypes will be uncovered, and indeed, genome-wide molecular studies have been used to search explicitly for epivariation in various contexts, particularly in plants (72, 121). We anticipate that similar approaches will eventually be applied to species without classical epivariable loci, such as *C. elegans* or *Drosophila melanogaster*, to broaden the catalog of epivariation across biology.

Transgenerational Inheritance Induced by Genetic Lesions

Although epialleles can arise spontaneously (146), they are more readily generated in response to abrupt genetic changes including deletion/mutation of epigenetic machinery or insertions of novel sequences such as transposable elements or transgenes. Together, these two approaches provide the experimental backbone of genetic interrogation of epigenetic phenomena. The machinery responsible for epigenetic silencing of, say, *b1* is revealed via deletion of genes involved in the various epigenetic silencing pathways (9), whereas *cis*-acting elements responsible for *FWA* silencing are identified via transgene dissections (28).

Mutations affecting the epigenetic machinery. In addition to its utility in dissecting specific epigenetic models, deletion of epigenetic regulators results in genome-wide generation of epialleles whose fate, following restoration of the relevant epigenetic machinery, provides an interesting probe for *cis* and *trans* determinants of epigenetic stability. For example, *Arabidopsis* mutants lacking the Ddm1 chromatin remodeler exhibit widespread deficits in DNA methylation (149). Following restoration of Ddm1 function via crosses to wild-type plants, roughly one-half to two-thirds of Ddm1-specific hypomethylated repeats were remethylated within two to three generations (143). The remainder were hypomethylated even eight generations later and exhibited Mendelian segregation in genetic crosses. At DMRs that were remethylated, rapid remethylation was driven by 24-nt RNA-directed DNA methylation, while those loci that were not targeted by preexisting RNAs in this pathway were, in contrast, susceptible to epigenetic instability. The fate of potential epialleles resulting from genetic manipulation varies dramatically between different systems. Epialleles are often rapidly erased following restoration of silencing machinery, as, for example, heterochromatin in budding yeast is rapidly restored after the Sir silencing complex is reintroduced to *sir* mutants (70).

In several cases, persistent effects of ancestral mutations have been identified for gross organismal phenotypes, without the underlying causative epiallele(s) being immediately apparent. For example, *C. elegans* lacking the COMPASS complex, responsible for H3 lysine 4 trimethylation, exhibit prolonged lifespan, and increased longevity persists for three to four generations following reintroduction of functional COMPASS subunits (42). Transgenerational modulation of lifespan could be passed down through either germline, and interestingly, the enhanced lifespan of progeny requires the presence of an intact germline. Restoration of COMPASS activity resulted in global recovery of H3K4me3 levels, suggesting either that increased longevity might result from inheritance of K4 methylation changes only at a subset of genomic loci or that some other epigenetic information carrier (e.g., small RNAs, or DNA modification) carries the memory of the COMPASS-deficient state. Genetic manipulation of germline H3K4 methylation also affects future generations in mammals. In this case, germline overexpression of the H3K4 demethylase Lsd1 resulting in decreased H3K4me2 in sperm caused impaired development not only in F1 offspring, but also in the F2 generation (133). In both worms and mice, the mechanistic basis for inheritance of an altered phenotype is unknown, as chromatin manipulations not only affect germline histone modifications, but also result in altered RNA levels and (in mammals) cytosine methylation patterns.

Epigenetic responses to novel sequences. In addition to loss of epigenetic machinery, novel DNA sequences introduced into the genome can also induce lasting epigenetic states. Epigenetic silencing of novel sequence elements represents a defense mechanism against transposon mobilization, and these defense systems are recruited to silence transgenes in response to laboratory genome engineering. In plants, a wide variety of transgenes—including those of viral origin

(83)—undergo posttranscriptional gene silencing, in which transgene-derived RNA is degraded (17, 91). Not only are transgenes targeted by this mechanism, but endogenous genes with homology to transgenes can also be silenced. As a particularly famous example, attempts to engineer more colorful petunias via overexpression of chalcone synthetase genes resulted, paradoxically, in colorless flowers (98). Many similar failures have been documented in a variety of organisms. Transgenes in mammals, for example, often become imprinted. Such failures provided early hints of the genome defense mechanism, which was termed RNAi in the classic study in *C. elegans* by Fire et al. (36). Here, Mello and colleagues showed that RNAi is induced by double-stranded RNA, that silencing activity can travel between cells, and that silencing of endogenous genes can in some cases be inherited epigenetically for four to five generations. Although RNAi in worms was originally described as a response to injected double-stranded RNA, more recent studies have used germline-expressed transgenes to induce heritable RNAi and begin to dissect the sequence features required for this process (10, 23, 130).

The mechanistic basis for self/nonself discrimination in the germline differs substantially between different species and is not completely understood in most cases. In some cases, transgenes and transposons are sensed or targeted according to features intrinsic to their life cycle. For example, meiotic silencing of unpaired DNA naturally identifies novel genomic insertions on the basis of their presence in only one of two homologous chromosomes (131), whereas the production of double-stranded RNA during replication of many retroelements accounts for the special role of double-stranded RNA in induction of RNAi (36, 114). Similarly, LTR retroelements almost universally utilize endogenous tRNAs during their life cycle. tRNAs serve as primers for reverse transcriptase (88), and tRNA cleavage products can suppress LTR endogenous retroviruses (89, 123). More generally, many of the complex mechanistic features of eukaryotic gene regulation, such as RNA capping, splicing, etc., may have evolved in part to enable genomes to identify exogenous RNAs with unexpected structures (86). For example, siRNAs in *Cryptococcus neoformans*, which exhibits a rather narrow distribution of intron lengths, are produced in response to spliceosome stalling on transcripts with abnormal intron lengths (33).

In addition to these innate defense systems, germline expression of piRNAs in animals is often described as an adaptive genomic immune system. For example, in *Drosophila*, special repeat-rich genomic loci provide the templates for generation of antitransposon piRNAs (20). Successful transposons will at some frequency insert into these clusters, and subsequent production of piRNAs targeting the repeat element in question can then direct silencing of dispersed transposon copies and prevent additional infections (75). A distinct adaptive surveillance mechanism is found in *Arabidopsis* pollen, where transposons are derepressed and thereby revealed in the somatic companion nucleus—the vegetative nucleus—resulting in processing of transposon mRNAs into 21-nt siRNAs, which are then shipped into sperm nuclei (90, 134). In *C. elegans*, the adaptive surveillance system appears to remember the germline transcriptome, and the protective Argonaute protein CSR1, which carries RdRP-derived small RNAs (22G-RNAs), targets all germline-expressed mRNAs, thereby allowing the next generation to identify novel RNAs that do not match this memory cache.

Transgenerational Effects of Environmental Perturbations

The increasing appreciation that germline epigenetic machinery can transmit information from one generation to the next, coupled with the fact that these pathways are also central to various environmental responses in somatic cells, has led to a resurrection of the largely discredited idea that an organism's environment can induce potentially adaptive phenotypes that manifest for multiple generations of offspring. However, although the idea of inheritance of acquired characters

is associated with early evolutionary theory, the majority of environmentally induced epigenetic states do not persist over long timescales and, thus, are unlikely to play roles in macroevolutionary processes. For example, even in plants where epialleles are generally more robustly inherited than in animals, the widespread cytosine methylation changes observed in response to hyperosmotic stress in *Arabidopsis* (154) or phosphate deprivation in rice (126) are transient, being erased either immediately after returning plants to control conditions, or in F1 offspring. With few exceptions, even when ancestral environments do appear to have persistent effects on offspring, these effects are lost within three to five generations. Thus, in our view, most if not all Lamarckian inheritance patterns are best considered to be special cases of plasticity in which organismal responses to the environment not only occur in the individual experiencing a particular environment, but also persist for one or two generations of offspring, thereby potentially providing beneficial information about prevailing conditions (65, 66, 79). However, they are not stable enough to be major contributors to macroevolution.

A burgeoning number of studies in a wide range of species have documented effects of ancestral environments that persist for one to two generations. Perhaps the most commonly reported intergenerational environmental effects are induced by conditions chosen to interfere with specific epigenetic information carriers. In mammals, manipulation of methyl donor vitamin levels—central to production of substrates required for cytosine and histone methylation—has been reported to skew offspring coat color in A^{vy} reporter animals (152), whereas hypomorphic *Mtrr* mutations that affect one carbon metabolism result in congenital malformations for multiple (up to four or five) generations of offspring (103). Similarly, as discussed in more detail below, heat shock interferes with transposon/transgene silencing mechanisms in many organisms, resulting in phenotypic effects that persist for several generations. In addition to environments chosen to globally disrupt a given epigenetic pathway, many studies report inter- or transgenerational effects of perturbations (e.g., endocrine disruptors, nicotine) that target other types of signaling pathway not central to the epigenetic machinery. Below, we discuss examples of both types of paradigm, focusing on transgenerational effects of heat shock across many species and on paternal dietary and stress paradigms in mammals.

Transgenerational effects of heat shock in model organisms. Since McClintock's discovery and analysis of transposable elements, exposure to environmental stressors has been known to alter the activity of repetitive elements in the genome (92). Among the most common stressors experienced in the wild is high temperature, which causes impaired epigenetic silencing in multiple model organisms. What makes epigenetic silencing pathways so susceptible to heat stress? Given the centrality of small RNAs in repeat recognition/targeting, one possibility is that this process is crippled owing to weakened base-pairing interactions at elevated temperatures. However, temperature affects many additional aspects of transposon control, in some cases in species-specific ways. For example, the transposon most dramatically induced by heat stress in *Arabidopsis* is the Copia-class retroelement ONSEN (for hot springs), which is temperature dependent because of a heat response element in its LTR (27, 64). In *Drosophila*, loss of heterochromatin at elevated temperatures was linked to signal-dependent phosphorylation of dATF-2, which has been implicated in heterochromatin nucleation (127). Other mechanisms may be more general, as many epigenetic regulators are known clients of heat shock proteins, which can be overwhelmed during heat stress.

Despite the potentially disparate mechanisms causing impaired epigenetic silencing at elevated temperatures, heat stress in several species not only affects the exposed generation, but also causes persistent phenotypic effects in subsequent generations. In plants, heat stress induces changes in morphology that persist for a handful of generations (64, 94, 139). In flies and worms, exposure to elevated temperatures results in impaired repeat silencing. In worms, this is

measured as loss of transgene silencing (77), whereas in flies, this is reflected in altered silencing of heterochromatin-adjacent reporters in position-effect variegation (40, 52). In both cases, loss of heterochromatin-based silencing persists for several generations after the heat stress. In flies, increased red pigmentation (resulting from impaired silencing of the reporter gene *white*) is observed for up to five generations and can be transmitted paternally (127). Notably, in both cases, stressing multiple generations results in more persistent epigenetic defects (77, 127). Interestingly, transgene repression in worms appears to be transmitted in *cis*, whereas heat shock effects in flies operate in *trans*: Offspring of a cross between a heat-stressed male and a female transmitting a *wh* reporter exhibit *wh* derepression, even though the affected reporter was not present in the stressed animal.

Paternal effects in mammals. Although there is little evidence for long-term inheritance of epialleles in mammals, interest in human health and disease has motivated a wide range of exposure studies (primarily in rodent models) to address multigenerational consequences of an individual's exposure history. It is unsurprising that maternal environment affects offspring. For example, drinking during pregnancy causes fetal alcohol syndrome in human children, which reflects direct action of the environment on the developing fetus. Although some oocyte and embryo transfer studies rigorously separate oocyte and fetal effects (60, 120), such studies are fairly rare (112). By contrast, males often contribute little more than sperm (and associated materials such as seminal fluids) upon mating, making mechanistic dissection of paternal effects relatively straightforward, at least conceptually. Whereas robust intergenerational effects of paternal environment on the F1 generation have been reported in response to a range of conditions in mammals, phenotypes that persist to F2 or F3 generations are less common. In most (but not all) cases, phenotypes reported in F1 offspring are either absent or quantitatively far diminished in F2 and F3 generations.

Broadly speaking, reported paternal-effect paradigms in mammals can be separated into three categories: dietary interventions, stress exposures, and toxin exposures (80, 111). Dietary interventions focus primarily on high-fat diets, low-protein diets, and caloric restriction, all of which affect metabolic parameters—glucose control and lipid metabolism, primarily—in offspring (4, 26, 69, 99). Paternal stress conditions include social defeat, maternal separation, and chronic unpredictable stress, and these interventions have been linked to altered cortisol release, metabolism, and blood-brain barrier function in the next generation (13, 37, 118). Finally, toxins and bioactive compounds used in paternal-effect studies range from endocrine disruptors (vinclozolin, BPA, etc.), to carbon tetrachloride, to drugs of abuse including nicotine and cocaine (5, 145, 147, 156). In general, paternal-effect studies involve perturbations applied during one of two timeframes: (*a*) from weaning until sexual maturity, essentially mimicking late childhood and early adulthood, and (*b*) during fetal development. In the latter, pregnant females are exposed to a condition such as starvation (69) or injection with high doses of endocrine disruptors (5) and male offspring that were exposed in utero are used to sire an F1 generation (the F2 generation relative to the injected/starved pregnant female).

What is the molecular mechanism for information transfer from father to child in these paradigms? Males can influence their offspring phenotype via nongermline mechanisms including seminal fluid, cryptic maternal effects, transfer of the microbiome, etc. (31, 111). However, in a handful of studies, paternal exposures affected offspring produced using purified gametes (29, 32, 60, 129), consistent with the sperm epigenome being responsible for reprogramming offspring. A diverse and at times conflicting set of epigenetic alterations—including changes to sperm chromatin, cytosine methylation, and small RNA payload—has been reported to occur in response to paternal exposures. Although too broad to fully cover here, we simply point out three important issues. First, it is often difficult to address rigorously whether a given epigenetic modification causes the development of the offspring phenotype, particularly for locus-specific cytosine methylation

and chromatin changes. Some progress has been made in the case of sperm RNA contents, where microinjection of either purified sperm RNAs or synthetic RNA mixtures has been used in certain paradigms to partially reproduce paternally induced phenotypes in offspring (29, 37, 41, 118, 129). Second, how does a given epigenetic change cause the development of the eventual phenotype? It is unclear why zygotic levels of microRNA-29, say, would cause metabolic problems in later life. Related to this issue, fairly distinct RNAs—individual microRNAs (41) versus gel-purified sperm tRNA fragments (29), for example—can alter the same phenotype (glucose control). Such findings suggest that multiple molecular changes convergently result in some blunt developmental change (e.g., altered preimplantation growth rate) that results in a pleiotropic phenotype in later life. Third, as far as we can tell, in no system has the signaling pathway that causes specific epigenetic changes in mature sperm been identified. How does paternal stress alter levels of specific microRNAs in sperm (37, 117, 118), or why does starvation induce alterations in cytosine methylation at *Kcnj11* (109)? Addressing these and related questions will be central issues in coming years.

SUMMARY AND OUTSTANDING QUESTIONS

Decades of genetic studies of various key model systems—paramutation, position effect variegation, transgene silencing, RNAi, imprinting—have identified and delineated a surprisingly conserved set of core epigenetic transmission pathways whose original goal was presumably discrimination between self and nonself and that are deployed to a diverse set of regulatory ends in different species. Although the molecular machinery of these pathways has been extensively cataloged, both core and species-specific components continue to be discovered. Germline dynamics of the epigenome are also moderately well-characterized for some epigenomes—cytosine methylation across the mammalian life cycle, for example—whereas other epigenetic marks have been far more challenging to study in limiting cell populations. More obscure are the rules that distinguish memorable from unstable epialleles: How are some genomic loci protected from germline reprogramming? And, if such a question is meaningful, why? How do stable epialleles contribute to phenotypic evolution in plants?

Finally, a burgeoning set of studies has resurrected the once-heretical idea that ancestral environments can affect future generations, but none of these paradigms are understood mechanistically. How does the environment affect the germ cell epigenome? How do germline epigenetic marks program a coherent phenotype in offspring? Are offspring phenotypes adaptive under ecologically relevant conditions? How much information is transmitted by the germline—how coarse-grained is the representation of the world provided by parents to their children? Medically, do the models implemented in rodents and other species also apply to humans, and can (and should) we manipulate the germline epigenome to predictable ends? These and other questions have a huge number of implications for evolution, developmental biology, and epidemiology.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank C. Conine for comments on a draft of this manuscript. Work in the Rando lab is supported by the National Institutes of Health R01HD080224, and A.B. is supported by a postdoctoral fellowship from Human Frontier Science Program (LT000857/2015-L).

LITERATURE CITED

1. Alabert C, Groth A. 2012. Chromatin replication and epigenome maintenance. *Nat. Rev. Mol. Cell Biol.* 13:153–67
2. Alcazar RM, Lin R, Fire AZ. 2008. Transmission dynamics of heritable silencing induced by double-stranded RNA in *Caenorhabditis elegans*. *Genetics* 180:1275–88
3. Amodeo AA, Jukam D, Straight AF, Skotheim JM. 2015. Histone titration against the genome sets the DNA-to-cytoplasm threshold for the *Xenopus* midblastula transition. *PNAS* 112:E1086–95
4. Anderson LM, Riffle L, Wilson R, Travlos GS, Lubomirski MS, Alvord WG. 2006. Preconceptional fasting of fathers alters serum glucose in offspring of mice. *Nutrition* 22:327–31
5. Anway MD, Cupp AS, Uzumcu M, Skinner MK. 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308:1466–69
6. Aravin AA, Hannon GJ, Brennecke J. 2007. The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. *Science* 318:761–64
7. Aravin AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, et al. 2008. A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. *Mol. Cell* 31:785–99
8. Arney KL, Bao S, Bannister AJ, Kouzarides T, Surani MA. 2002. Histone methylation defines epigenetic asymmetry in the mouse zygote. *Int. J. Dev. Biol.* 46:317–20
9. Arteaga-Vazquez MA, Chandler VL. 2010. Paramutation in maize: RNA mediated *trans*-generational gene silencing. *Curr. Opin. Genet. Dev.* 20:156–63
10. Ashe A, Sapetschnig A, Weick EM, Mitchell J, Bagijn MP, et al. 2012. piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. *Cell* 150:88–99
11. Autran D, Baroux C, Raissig MT, Lenormand T, Wittig M, et al. 2011. Maternal epigenetic pathways control parental contributions to *Arabidopsis* early embryogenesis. *Cell* 145:707–19
12. Bagijn MP, Goldstein LD, Sapetschnig A, Weick EM, Bouasker S, et al. 2012. Function, targets, and evolution of *Caenorhabditis elegans* piRNAs. *Science* 337:574–78
13. Bale TL. 2015. Epigenetic and transgenerational reprogramming of brain development. *Nat. Rev. Neurosci.* 16:332–44
14. Barton SC, Surani MA, Norris ML. 1984. Role of paternal and maternal genomes in mouse development. *Nature* 311:374–76
15. Bateson W, Pellew C. 1915. On the genetics of "rogues" among culinary peas (*Pisum sativum*). *J. Genet.* 5:15–36
16. Batista PJ, Ruby JG, Claycomb JM, Chiang R, Fahlgren N, et al. 2008. PRG-1 and 21U-RNAs interact to form the piRNA complex required for fertility in *C. elegans*. *Mol. Cell* 31:67–78
17. Baulcombe DC. 1996. Mechanisms of pathogen-derived resistance to viruses in transgenic plants. *Plant Cell* 8:1833–44
18. Bonnefoy E, Orsi GA, Couble P, Loppin B. 2007. The essential role of *Drosophila* HIRA for de novo assembly of paternal chromatin at fertilization. *PLOS Genet.* 3:1991–2006
19. Borges F, Martienssen RA. 2015. The expanding world of small RNAs in plants. *Nat. Rev. Mol. Cell Biol.* 16:727–41
20. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, et al. 2007. Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. *Cell* 128:1089–103
21. Brennecke J, Malone CD, Aravin AA, Sachidanandam R, Stark A, Hannon GJ. 2008. An epigenetic role for maternally inherited piRNAs in transposon silencing. *Science* 322:1387–92
22. Brink RA. 1958. Paramutation at the *R* locus in maize. *Cold Spring Harb. Symp. Quant. Biol.* 23:379–91
23. Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, et al. 2012. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. *Nature* 489:447–51
24. Calarco JP, Borges F, Donoghue MT, Van Ex F, Jullien PE, et al. 2012. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell* 151:194–205
25. Campos EI, Stafford JM, Reinberg D. 2014. Epigenetic inheritance: histone bookmarks across generations. *Trends Cell Biol.* 24:664–74
26. Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, et al. 2010. Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* 143:1084–96

27. Cavrak VV, Lettner N, Jamge S, Kosarewicz A, Bayer LM, Mittelsten Scheid O. 2014. How a retrotransposon exploits the plant's heat stress response for its activation. *PLOS Genet*. 10:e1004115
28. Chan SW, Zhang X, Bernatavichute YV, Jacobsen SE. 2006. Two-step recruitment of RNA-directed DNA methylation to tandem repeats. *PLOS Biol*. 4:e363
29. Chen Q, Yan M, Cao Z, Li X, Zhang Y, et al. 2016. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* 351:397–400
30. Coleman RT, Struhl G. 2017. Causal role for inheritance of H3K27me3 in maintaining the OFF state of a *Drosophila* HOX gene. *Science* 356:eaai8236
31. Curley JP, Mashoodh R, Champagne FA. 2010. Epigenetics and the origins of paternal effects. *Horm. Behav.* 59:306–14
32. Dias BG, Ressler KJ. 2014. Parental olfactory experience influences behavior and neural structure in subsequent generations. *Nat. Neurosci.* 17:89–96
33. Dumesic PA, Natarajan P, Chen C, Drinnenberg IA, Schiller BJ, et al. 2013. Stalled spliceosomes are a signal for RNAi-mediated genome defense. *Cell* 152:957–68
34. Erkek S, Hisano M, Liang CY, Gill M, Murr R, et al. 2013. Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. *Nat. Struct. Mol. Biol.* 20:868–75
35. Feng S, Jacobsen SE, Reik W. 2010. Epigenetic reprogramming in plant and animal development. *Science* 330:622–27
36. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391:806–11
37. Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, et al. 2014. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nat. Neurosci.* 17:667–69
38. Gehring M, Bubb KL, Henikoff S. 2009. Extensive demethylation of repetitive elements during seed development underlies gene imprinting. *Science* 324:1447–51
39. Ghildiyal M, Zamore PD. 2009. Small silencing RNAs: an expanding universe. *Nat. Rev. Genet.* 10:94–108
40. Gowen JW, Gay EH. 1933. Effect of temperature on eversporting eye color in *Drosophila melanogaster*. *Science* 77:312
41. Grandjean V, Fourre S, De Abreu DA, Derieppe MA, Remy JJ, Rassoulzadegan M. 2015. RNA-mediated paternal heredity of diet-induced obesity and metabolic disorders. *Sci. Rep.* 5:18193
42. Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, et al. 2011. Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature* 479:365–71
43. Gu W, Lee HC, Chaves D, Youngman EM, Pazour GJ, et al. 2013. CapSeq and CIP-TAP identify Pol II start sites and reveal capped small RNAs as *C. elegans* piRNA precursors. *Cell* 151:1488–500
44. Guerrero-Bosagna C, Skinner MK. 2014. Environmentally induced epigenetic transgenerational inheritance of male infertility. *Curr. Opin. Genet. Dev.* 26:79–88
45. Guo H, Zhu P, Yan L, Li R, Hu B, et al. 2014. The DNA methylation landscape of human early embryos. *Nature* 511:606–10
46. Gurdon JB. 2006. From nuclear transfer to nuclear reprogramming: the reversal of cell differentiation. *Annu. Rev. Cell Dev. Biol.* 22:1–22
47. Hackett JA, Sengupta R, Zylicz JJ, Murakami K, Lee C, et al. 2013. Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* 339:448–52
48. Hackett JA, Surani MA. 2013. DNA methylation dynamics during the mammalian life cycle. *Philos. Trans. R. Soc. B* 368:20110328
49. Haig D. 2004. Genomic imprinting and kinship: How good is the evidence? *Annu. Rev. Genet.* 38:553–85
50. Haig D, Wilkins JF. 2000. Genomic imprinting, sibling solidarity and the logic of collective action. *Philos. Trans. R. Soc. B* 355:1593–97
51. Hanna CW, Taudt A, Huang J, Gahurova L, Kranz A, et al. 2018. MLL2 conveys transcription-independent H3K4 trimethylation in oocytes. *Nat. Struct. Mol. Biol.* 25:73–82
52. Hartmann-Goldstein IJ. 1967. On the relationship between heterochromatization and variegation in *Drosophila*, with special reference to temperature-sensitive periods. *Genet. Res.* 10:143–59
53. Harvey ZH, Chen Y, Jarosz DF. 2017. Protein-based inheritance: epigenetics beyond the chromosome. *Mol. Cell* 69:195–202

54. Heard E, Martienssen RA. 2014. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* 157:95–109
55. Hermant C, Boivin A, Teysset L, Delmarre V, Asif-Laidin A, et al. 2015. Paramutation in *Drosophila* requires both nuclear and cytoplasmic actors of the piRNA pathway and induces *cis*-spreading of piRNA production. *Genetics* 201:1381–96
56. Hirsch S, Baumberger R, Grossniklaus U. 2012. Epigenetic variation, inheritance, and selection in plant populations. *Cold Spring Harb. Symp. Quant. Biol.* 77:97–104
57. Holoch D, Moazed D. 2015. RNA-mediated epigenetic regulation of gene expression. *Nat. Rev. Genet.* 16:71–84
58. Hsieh TF, Ibarra CA, Silva P, Zemach A, Eshed-Williams L, et al. 2009. Genome-wide demethylation of *Arabidopsis* endosperm. *Science* 324:1451–54
59. Huang X, Fejes Toth K, Aravin AA. 2017. piRNA biogenesis in *Drosophila melanogaster*. *Trends Genet.* 33:882–94
60. Huypens P, Sass S, Wu M, Dyckhoff D, Tschop M, et al. 2016. Epigenetic germline inheritance of diet-induced obesity and insulin resistance. *Nat. Genet.* 48:497–99
61. Ingouff M, Hamamura Y, Gourgues M, Higashiyama T, Berger F. 2007. Distinct dynamics of HISTONE3 variants between the two fertilization products in plants. *Curr. Biol.* 17:1032–37
62. Inoue A, Jiang L, Lu F, Suzuki T, Zhang Y. 2017. Maternal H3K27me3 controls DNA methylation-independent imprinting. *Nature* 547:419–24
63. Inoue A, Zhang Y. 2014. Nucleosome assembly is required for nuclear pore complex assembly in mouse zygotes. *Nat. Struct. Mol. Biol.* 21:609–16
64. Ito H, Gaubert H, Bucher E, Mirouze M, Vaillant I, Paszkowski J. 2011. An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature* 472:115–19
65. Jablonka E, Lamb MJ. 1989. The inheritance of acquired epigenetic variations. *J. Theor. Biol.* 139:69–83
66. Jablonka E, Lamb MJ. 1995. *Epigenetic Inheritance and Evolution: The Lamarckian Dimension*. Oxford, UK: Oxford Univ. Press
67. Jacobsen SE, Meyerowitz EM. 1997. Hypermethylated *SUPERMAN* epigenetic alleles in *Arabidopsis*. *Science* 277:1100–3
68. Jiang L, Zhang J, Wang JJ, Wang L, Zhang L, et al. 2013. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell* 153:773–84
69. Jimenez-Chillaron JC, Isganaitis E, Charalambous M, Gesta S, Pentinat-Pelegrin T, et al. 2009. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. *Diabetes* 58:460–68
70. Katan-Khaykovich Y, Struhl K. 2005. Heterochromatin formation involves changes in histone modifications over multiple cell generations. *EMBO J.* 24:2138–49
71. Kaufman PD, Rando OJ. 2010. Chromatin as a potential carrier of heritable information. *Curr. Opin. Cell Biol.* 22:284–90
72. Kawakatsu T, Huang SC, Jupe F, Sasaki E, Schmitz RJ, et al. 2016. Epigenomic diversity in a global collection of *Arabidopsis thaliana* accessions. *Cell* 166:492–505
73. Kawashima T, Berger F. 2014. Epigenetic reprogramming in plant sexual reproduction. *Nat. Rev. Genet.* 15:613–24
74. Kelly WG, Aramayo R. 2007. Meiotic silencing and the epigenetics of sex. *Chromosome Res.* 15:633–51
75. Khurana JS, Wang J, Xu J, Koppetsch BS, Thomson TC, et al. 2011. Adaptation to *P* element transposon invasion in *Drosophila melanogaster*. *Cell* 147:1551–63
76. Klattenhoff C, Xi H, Li C, Lee S, Xu J, et al. 2009. The *Drosophila* HP1 homolog Rhino is required for transposon silencing and piRNA production by dual-strand clusters. *Cell* 138:1137–49
77. Klosin A, Casas E, Hidalgo-Carcedo C, Vavouri T, Lehner B. 2017. Transgenerational transmission of environmental information in *C. elegans*. *Science* 356:320–23
78. Kobayashi H, Sakurai T, Imai M, Takahashi N, Fukuda A, et al. 2012. Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks. *PLOS Genet.* 8:e1002440
79. Kussell E, Leibler S. 2005. Phenotypic diversity, population growth, and information in fluctuating environments. *Science* 309:2075–78

80. Lane M, Robker RL, Robertson SA. 2014. Parenting from before conception. *Science* 345:756–60
81. Lee HC, Gu W, Shirayama M, Youngman E, Conte D Jr., Mello CC. 2013. *C. elegans* piRNAs mediate the genome-wide surveillance of germline transcripts. *Cell* 150:78–87
82. Li XZ, Roy CK, Dong X, Bolcun-Filas E, Wang J, et al. 2013. An ancient transcription factor initiates the burst of piRNA production during early meiosis in mouse testes. *Mol. Cell* 50:67–81
83. Lindbo JA, Silva-Rosales L, Proebsting WM, Dougherty WG. 1993. Induction of a highly specific antiviral state in transgenic plants: implications for regulation of gene expression and virus resistance. *Plant Cell* 5:1749–59
84. Luo GZ, Blanco MA, Greer EL, He C, Shi Y. 2015. DNA N^6-methyladenine: a new epigenetic mark in eukaryotes? *Nat. Rev. Mol. Cell Biol.* 16:705–10
85. Lyon MF. 1961. Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature* 190:372–73
86. Madhani HD. 2013. The frustrated gene: origins of eukaryotic gene expression. *Cell* 155:744–49
87. Malone CD, Hannon GJ. 2009. Small RNAs as guardians of the genome. *Cell* 136:656–68
88. Marquet R, Isel C, Ehresmann C, Ehresmann B. 1995. tRNAs as primer of reverse transcriptases. *Biochimie* 77:113–24
89. Martinez G, Choudury SG, Slotkin RK. 2017. tRNA-derived small RNAs target transposable element transcripts. *Nucleic Acids Res.* 45:5142–52
90. Martinez G, Panda K, Kohler C, Slotkin RK. 2016. Silencing in sperm cells is directed by RNA movement from the surrounding nurse cell. *Nat. Plants* 2:16030
91. Matzke MA, Matzke A. 1995. How and why do plants inactivate homologous (trans)genes? *Plant Physiol.* 107:679–85
92. McClintock B. 1984. The significance of responses of the genome to challenge. *Science* 226:792–801
93. McGrath J, Solter D. 1984. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 37:179–83
94. Migicovsky Z, Yao Y, Kovalchuk I. 2014. Transgenerational phenotypic and epigenetic changes in response to heat stress in *Arabidopsis thaliana*. *Plant Signal. Behav.* 9:e27971
95. Miska EA, Ferguson-Smith AC. 2016. Transgenerational inheritance: models and mechanisms of non-DNA sequence-based inheritance. *Science* 354:59–63
96. Morgan HD, Sutherland HG, Martin DI, Whitelaw E. 1999. Epigenetic inheritance at the agouti locus in the mouse. *Nat. Genet.* 23:314–18
97. Nakamura T, Liu YJ, Nakashima H, Umehara H, Inoue K, et al. 2012. PGC7 binds histone H3K9me2 to protect against conversion of 5mC to 5hmC in early embryos. *Nature* 486:415–19
98. Napoli C, Lemieux C, Jorgensen R. 1990. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell* 2:279–89
99. Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, Morris MJ. 2010. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* 467:963–66
100. Okada T, Endo M, Singh MB, Bhalla PL. 2005. Analysis of the histone H3 gene family in *Arabidopsis* and identification of the male-gamete-specific variant *AtMGH3*. *Plant J.* 44:557–68
101. Ooi SL, Henikoff S. 2007. Germline histone dynamics and epigenetics. *Curr. Opin. Cell Biol.* 19:257–65
102. Ooi SL, Priess JR, Henikoff S. 2006. Histone H3.3 variant dynamics in the germline of *Caenorhabditis elegans*. *PLOS Genet.* 2:e97
103. Padmanabhan N, Jia D, Geary-Joo C, Wu X, Ferguson-Smith AC, et al. 2013. Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. *Cell* 155:81–93
104. Paszkowski J, Grossniklaus U. 2011. Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Curr. Opin. Plant Biol.* 14:195–203
105. Peng H, Shi J, Zhang Y, Zhang H, Liao S, et al. 2012. A novel class of tRNA-derived small RNAs extremely enriched in mature mouse sperm. *Cell Res.* 22:1609–12
106. Potok ME, Nix DA, Parnell TJ, Cairns BR. 2013. Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell* 153:759–72
107. Ptashne M. 2007. On the use of the word 'epigenetic.' *Curr. Biol.* 17:R233–36
108. Quadrana L, Colot V. 2016. Plant transgenerational epigenetics. *Annu. Rev. Genet.* 50:467–91
109. Radford EJ, Ito M, Shi H, Corish JA, Yamazawa K, et al. 2014. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science* 345:1255903

110. Raissig MT, Baroux C, Grossniklaus U. 2011. Regulation and flexibility of genomic imprinting during seed development. *Plant Cell* 23:16–26
111. Rando OJ. 2012. Daddy issues: paternal effects on phenotype. *Cell* 151:702–8
112. Rando OJ, Simmons RA. 2015. I'm eating for two: parental dietary effects on offspring metabolism. *Cell* 161:93–105
113. Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, Cuzin F. 2006. RNA-mediated non-Mendelian inheritance of an epigenetic change in the mouse. *Nature* 441:469–74
114. Ratcliff F, Harrison BD, Baulcombe DC. 1997. A similarity between viral defense and gene silencing in plants. *Science* 276:1558–60
115. Raychaudhuri N, Dubruille R, Orsi GA, Bagheri HC, Loppin B, Lehner CF. 2012. Transgenerational propagation and quantitative maintenance of paternal centromeres depends on Cid/Cenp-A presence in *Drosophila* sperm. *PLOS Biol*. 10:e1001434
116. Rinn JL, Chang HY. 2012. Genome regulation by long noncoding RNAs. *Annu. Rev. Biochem*. 81:145–66
117. Rodgers AB, Morgan CP, Bronson SL, Revello S, Bale TL. 2013. Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *J. Neurosci.* 33:9003–12
118. Rodgers AB, Morgan CP, Leu NA, Bale TL. 2015. Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. *PNAS* 112:13699–704
119. Santenard A, Ziegler-Birling C, Koch M, Tora L, Bannister AJ, Torres-Padilla ME. 2010. Heterochromatin formation in the mouse embryo requires critical residues of the histone variant H3.3. *Nat. Cell Biol.* 12:853–62
120. Sasson IE, Vitins AP, Mainigi MA, Moley KH, Simmons RA. 2015. Pre-gestational versus gestational exposure to maternal obesity differentially programs the offspring in mice. *Diabetologia* 58:615–24
121. Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA, et al. 2011. Transgenerational epigenetic instability is a source of novel methylation variants. *Science* 334:369–73
122. Schoft VK, Chumak N, Mosiolek M, Slusarz L, Komnenovic V, et al. 2009. Induction of RNA-directed DNA methylation upon decondensation of constitutive heterochromatin. *EMBO Rep*. 10:1015–21
123. Schorn AJ, Gutbrod MJ, LeBlanc C, Martienssen R. 2017. LTR-retrotransposon control by tRNA-derived small RNAs. *Cell* 170:61–71.e11
124. Schuettengruber B, Chourrout D, Vervoort M, Leblanc B, Cavalli G. 2007. Genome regulation by polycomb and trithorax proteins. *Cell* 128:735–45
125. Scott RJ, Spielman M, Bailey J, Dickinson HG. 1998. Parent-of-origin effects on seed development in *Arabidopsis thaliana*. *Development* 125:3329–41
126. Secco D, Wang C, Shou H, Schultz MD, Chiarenza S, et al. 2015. Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. *eLife* 4:e09343
127. Seong KH, Li D, Shimizu H, Nakamura R, Ishii S. 2011. Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell* 145:1049–61
128. Serio TR, Lindquist SL. 1999. [*PSI+*]: an epigenetic modulator of translation termination efficiency. *Annu. Rev. Cell Dev. Biol.* 15:661–703
129. Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, et al. 2016. Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. *Science* 351:391–96
130. Shirayama M, Seth M, Lee HC, Gu W, Ishidate T, et al. 2012. piRNAs initiate an epigenetic memory of nonself RNA in the *C. elegans* germline. *Cell* 150:65–77
131. Shiu PK, Metzenberg RL. 2002. Meiotic silencing by unpaired DNA: properties, regulation and suppression. *Genetics* 161:1483–95
132. Shivaprasad PV, Dunn RM, Santos BA, Bassett A, Baulcombe DC. 2012. Extraordinary transgressive phenotypes of hybrid tomato are influenced by epigenetics and small silencing RNAs. *EMBO J.* 31:257–66
133. Siklenka K, Erkek S, Godmann M, Lambrot R, McGraw S, et al. 2015. Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science* 350:aab2006
134. Slotkin RK, Vaughn M, Borges F, Tanurdzic M, Becker JD, et al. 2009. Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* 136:461–72
135. Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, et al. 2012. A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* 484:339–44

136. Smith ZD, Shi J, Gu H, Donaghey J, Clement K, et al. 2017. Epigenetic restriction of extraembryonic lineages mirrors the somatic transition to cancer. *Nature* 549:543–47
137. Stam M, Belele C, Dorweiler JE, Chandler VL. 2002. Differential chromatin structure within a tandem array 100 kb upstream of the maize *b1* locus is associated with paramutation. *Genes Dev*. 16:1906–18
138. Stroud H, Greenberg MV, Feng S, Bernatavichute YV, Jacobsen SE. 2013. Comprehensive analysis of silencing mutants reveals complex regulation of the *Arabidopsis* methylome. *Cell* 152:352–64
139. Suter L, Widmer A. 2013. Environmental heat and salt stress induce transgenerational phenotypic changes in *Arabidopsis thaliana*. *PLOS ONE* 8:e60364
140. Svoboda P, Flemr M. 2010. The role of miRNAs and endogenous siRNAs in maternal-to-zygotic reprogramming and the establishment of pluripotency. *EMBO Rep*. 11:590–97
141. Takahashi K, Yamanaka S. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–76
142. Tang WW, Kobayashi T, Irie N, Dietmann S, Surani MA. 2016. Specification and epigenetic programming of the human germ line. *Nat. Rev. Genet.* 17:585–600
143. Teixeira FK, Heredia F, Sarazin A, Roudier F, Boccara M, et al. 2009. A role for RNAi in the selective correction of DNA methylation defects. *Science* 323:1600–4
144. Vagin VV, Sigova A, Li C, Seitz H, Gvozdev V, Zamore PD. 2006. A distinct small RNA pathway silences selfish genetic elements in the germline. *Science* 313:320–24
145. Vallaster MP, Kukreja S, Bing XY, Ngolab J, Zhao-Shea R, et al. 2017. Paternal nicotine exposure alters hepatic xenobiotic metabolism in offspring. *eLife* 6:e24771
146. van der Graaf A, Wardenaar R, Neumann DA, Taudt A, Shaw RG, et al. 2015. Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. *PNAS* 112:6676–81
147. Vassoler FM, White SL, Schmidt HD, Sadri-Vakili G, Pierce RC. 2013. Epigenetic inheritance of a cocaine-resistance phenotype. *Nat. Neurosci.* 16:42–47
148. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI, Martienssen RA. 2002. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 297:1833–37
149. Vongs A, Kakutani T, Martienssen RA, Richards EJ. 1993. *Arabidopsis thaliana* DNA methylation mutants. *Science* 260:1926–28
150. Wang X, Moazed D. 2017. DNA sequence-dependent epigenetic inheritance of gene silencing and histone H3K9 methylation. *Science* 356:88–91
151. Watanabe T, Takeda A, Tsukiyama T, Mise K, Okuno T, et al. 2006. Identification and characterization of two novel classes of small RNAs in the mouse germline: retrotransposon-derived siRNAs in oocytes and germline small RNAs in testes. *Genes Dev*. 20:1732–43
152. Waterland RA, Jirtle RL. 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Cell. Biol.* 23:5293–300
153. Weigel D, Colot V. 2012. Epialleles in plant evolution. *Genome Biol.* 13:249
154. Wibowo A, Becker C, Marconi G, Durr J, Price J, et al. 2016. Hyperosmotic stress memory in *Arabidopsis* is mediated by distinct epigenetically labile sites in the genome and is restricted in the male germline by DNA glycosylase activity. *eLife* 5:e13546
155. Yuan S, Schuster A, Tang C, Yu T, Ortogero N, et al. 2016. Sperm-borne miRNAs and endo-siRNAs are important for fertilization and preimplantation embryonic development. *Development* 143:635–47
156. Zeybel M, Hardy T, Wong YK, Mathers JC, Fox CR, et al. 2012. Multigenerational epigenetic adaptation of the hepatic wound-healing response. *Nat. Med.* 18:1369–77
157. Zierhut C, Jenness C, Kimura H, Funabiki H. 2014. Nucleosomal regulation of chromatin composition and nuclear assembly revealed by histone depletion. *Nat. Struct. Mol. Biol.* 21:617–25
158. Zilberman D, Cao X, Jacobsen SE. 2003. *ARGONAUTE4* control of locus-specific siRNA accumulation and DNA and histone methylation. *Science* 299:716–19